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said gene comprising coding sequences encoding for two or more enzymes and said gene being capable of being expressed in the cells of the transgenic plant.

30. A transgenic plant which harbors in its cells a chimaeric gene comprising;

(a) a promoter operably linked to

(b) a deoxyribonucleic acid fragment which comprises a coding sequence the product of which causes modification of the amount of metabolic intermediate in glycolysis or in a pathway for the synthesis or degradation of starch, sucrose or reducing sugar.

Cancel claims 1, 9-12 and 17-19.

R E M A R K S

Claims 1, 9-12 and 17-19 have been cancelled and new claims 27-30 entered. The new claims find support throughout the specification; see for example original claim 2.

Claims 2-4, 7-8, 13-16 and 20-30 are active in the application.

The specification has been amended as required in the Office Action at page 2.

A copy of Burrell's declaration filed 25 March 1993 in parent application Serial No. 07/991,451 is enclosed herewith as requested in the Office Action.

Claims 2, 13 and 20 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for the reasons set forth in the Office Action. The claims have been amended as suggested in the Office Action. Reconsideration of the rejection is requested.

Claims 21-26 stand rejected under 35 U.S.C. 112, fourth paragraph, as being of improper dependent form for the reasons set forth in the Office Action. The amendment now made is in accordance with the Examiner's suggestions and it is believed they remove the objections. Reconsideration of the rejection is requested.

Claims 2-4, 7-8, 13-16, 19 and 21-26 are rejected under 35 U.S.C. 112, first paragraph. It is alleged that the disclosure is enabling only for claims limited to a process for the introduction of a gene encoding either phosphofructokinase or adenine diphosphoglucose pyrophosphorylase into the genome of a plant cell. Reconsideration of the rejection is requested for the following reasons.

The claims concern processes for the preparation of transgenic plants by incorporating into a host plant, a chimaeric gene which includes the DNA comprising the

coding sequence encoding for an enzyme selected from the group consisting of:

1. adenine diphosphoglucose pyrophosphorylase
(hereinafter referred to as ("ADPG"));
2. acid invertase (hereinafter referred to as
"AI");
3. starch synthase;
4. sucrose synthase;
5. pyruvate kinase;
6. 6-phosphofructokinase (pyrophosphate); and
7. sucrose phosphate synthetase.

The parent application, now issued as U.S. Patent 5,387,756, concerned the DNA fragment which encoded for the enzyme phosphofructokinase for a total of 8 enzymes.

These 8 enzymes, although chemically diverse, are all related in that they function in plant cells to modify the amount of metabolic intermediate in glycolysis or in a pathway for the synthesis or degradation of starch, sucrose or a reducing sugar. The relationship of the 8 enzymes in affecting glycolysis has been set forth in the schematic diagram of Figure 1 in the drawings of applicants' specification. This relationship is well known to the skilled artisan; see for example the Biochemistry of Plants, Vol. 14, Ed. J. Preiss (especially Chapters 1 and 6).

The 8 enzymes are all well known, as are the DNA sequences which encode for their expression in plants. this has been described and set forth in applicants' specification at page 5, second full paragraph and third paragraph bridging page 6.

In the Office Action, it is said that:

"The specification only demonstrates the utilization of a gene encoding the glycolytic enzyme phosphofructokinase for plant transformation. In addition, the Burrell declaration submitted 25 March 1993 in parent application Serial No. 07/991,451 demonstrates the utilization of a gene encoding ADP glucose pyrophosphorylase. No guidance has been presented for the identification or isolation of other genes involved in glycolysis. It is unclear whether plant transformation with genes encoding other glycolytic enzymes would alter glycolysis and/or kill the transformed plant cells and plants (see e.g., page 3 of the specification, first full paragraph; page 3 of the ap Rees declaration filed 22 January 1993 in parent application Serial No. 07/991,451). It is also unclear whether plants transformed with more than one glycolytic gene would be adversely affected, given the "double dose" of glycolytic enzyme alteration."

However, as mentioned above, applicants' specification at pages 5 and 6 does indicate that the necessary DNA sequences have already been isolated and are

known. The general technique for identifying and isolating particular genes is certainly a known technique; see Khurshead et al., cited and of record in the application.

The real question posed by the Office Action is "the effects of said gene(s) on transformed plant cells and plants, given the unpredictability inherent in the process".

The question has been answered in respect to PFK and ADPG as acknowledged by the Examiner in the Office Action. In addition, before the filing of this application a Journal article had appeared (October, 1991) which indicates that tobacco plants have been transformed with acid invertase (AI) DNA sequences. The transgenic tobacco plants exhibited a new phenotype with accumulated carbohydrate, i.e.; an effect upon glycolysis as applicants herein have proposed. A copy of the article (Antje van Schaewen et al., The Embo Journal Vol. 9, No. 18, pp 3033-3044 [1990]) has been filed in the record. Although the publication is before the filing date of the present application, it is not a prior art reference within the meaning of 35 USC 102 or 103 since it does not antedate applicants' priority date under 35 USC 120. The publication fully supports and confirms enablement of applicants' invention in respect of 1) plants other than rice and potato and 2) DNA sequences coding for an enzyme

other than PFK and ADPG. In other words, the Journal article further confirms the broad embrace of applicants' claims 2-4, 7-8, 13-16 and 21-26, by showing that the skill of the art is such that the present invention is enabled with the specification teachings.

Of this argument, made previously in Applicants' parent application, it is said in the Office Action that: "von Schaewen et al. demonstrate the unpredictability inherent in the transformation of plants with genes encoding glycolytic enzymes. The transformation of tobacco resulted in dwarfing, bleaching and browning of leaves, and root stunting (see e.g., page 3037). The lack of such deleterious effects on plant health in the potato plants transformed with the PFK gene has been previously argued by Applicants as evidence of unexpected results. Respiration, i.e., glycolysis, was also inhibited (see e.g., page 3039, column 1, bottom paragraph). Furthermore, the transformation of a different plant species by von Schaewen et al. resulted in a completely different response to the introduction of a glycolytic gene, i.e., lack of appreciable change in phenotype or starch content (see e.g., page 3038, column 1, top paragraph; page 3039, column 2, bottom paragraph).

Applicants cited the von Schaewen et al. paper in support of their contention that it shows that the invention of Claim 2 has application in respect of genes

of specified group other than PFK and ADGPP and in respect of plants other than potato. Applicants remain of the belief that the paper fulfils that function. It shows that when tobacco and Arabidopsis plants are transformed with the invertase gene there results for both types of plant an increase in starch; See Tables III and IV of the reference.

As to the phenotypic changes in tobacco which were reported by von Schaewen et al. it is stated on page 3037 that considerable variation was observed between different transformants. Thus, in respect of height variations it is stated that some transformants almost reached the height of a wild-type plant, the latter height being given as about (i.e. variable) 150cm. Again, whereas bleaching and necrotic reactions were observed in respect of older leaves of some plants, for others bleaching was not accompanied by necrotic reactions.

The work of von Schaewen et al was of a strictly scientific nature. Genetic manipulation techniques were employed for the purpose of enhancing knowledge of sink-source relationships. However, to the skilled in the art addressee interested in a practical sense in effecting metabolic changes in plants, the major teaching of the von Schaewen et al. paper is that a process as per Claim 2 works effectively in respect of a sequence coding for acid invertase. In regard to tobacco, such person would

readily observe the variation in the phenotypic effects and that by virtue of such variation there was open to him the opportunity of selecting, according to his specific requirements, individuals which are little effected. From such individuals further individuals could be produced by cloning or crossing. It should also be noted that the said specific requirements may be many and various, and that according to some at least of these a phenotypic change, or a change present to a lesser degree, may be of little or no consequence, either in absolute terms or because the positive payoff of using the inventive method predominates.

Proposals have long been made for growing tobacco for the purpose of providing a source of food protein. As can be observed from Table 6B of the von Schaewen et al paper, there is a prospect of harvesting tobacco leaves having an enhanced level of protein.

In any event, applicants have by their own work confirmed the promise of von Schaewen et al.

Potato plants were transformed with a chimaeric gene comprising a patatin promoter, an antisense invertase coding sequence and a nos terminator. The bar chart attached as Exhibit A shows results, in terms on the Y-axis, of the ratio sucrose glucose + fructose. (On the chart "sem"=standard error of the mean).

The bar chart clearly shows that plants were obtained with a greatly increased ratio of sucrose to glucose + fructose. This is a commercially advantageous attribute in certain forms of potato processing. The person skilled in the art could readily select such plants and clonely multiply them, so to obtain commercial quantities thereof.

In a second procedure, potato was transformed with the chimaeric gene as above described and nine independent transformed lines were grown in a paired experiment with each line replicated ten times. As is shown by the results table below, the reducing sugar content, as represented by the content of glucose, is significantly less in the transgenic plants than in control plants, whereas in the transgenic plants the sucrose-glucose ratio is significantly increased relative to the control plants.

Line	Glucose mg/g fr wt Transgenic	Control	P	Ratio Sucrose:Glucose Trangenic	Control
2273	0.626	1.238	0.002	2.57	0.73
2062	0.363	0.749	0.006	4.26	2.19
2134	0.739	1.505	0.01	2.15	0.62
2263	0.923	1.299	0.015	2.24	1.22
2295	1.208	2.095	0.015	1.28	0.71
2042	1.499	2.054	0.024	0.98	0.56
2016	0.832	1.248	0.034	1.35	0.83
2294	0.996	1.418	0.035	1.15	0.67
2028	1.118	1.394	0.047	1.00	0.89

[In the results Table P is Fischers Probability. statistical difference is generally accepted if P is less than 0.05].

A field trial was conducted with potatoes transformed with the sequence for sucrose synthase in a chimaeric gene which also comprised a patatin promoter and a nos terminator. the trial was of a randomized block design and there were three replicates. An analysis of variance in regard to the specific gravity (an indicator of starch content) for the three lines indicates that they were significantly different from controls (standard error of difference = 0.0075).

Line	Specific Gravity
89	1.168
36	1.145
52	1.137
Control	1.117

Thus, the applicability of the invention has been shown for four plants (potato, rice, tobacco and Arabidopsis thaliana) and in respect of four of the enzymes.

For the above reasons, it is seen that the von Schaewen et al. paper evidences wide applicability of the invention and that it rebuts any position of unpredictability. Phenotypic reactions are a separate issue from workability (starch did increase). Furthermore, as outlined above, such reactions may, in particular circumstances, be acceptable, may be rectified or may be avoided.

It is clear that undue experimentation is not required, to identify and isolate the gene or genes which

encode any other glycolytic enzyme, and to evaluate the effects of said gene(s) on transformed plant cells and plants.

It is also said in the Office Action that:

"Furthermore, ap-Rees et al. teach that only three out of many glycolytic or starch metabolic enzymes are cold-labile in potato (see e.g., paragraph bridging pages 377 and 378; page 384; pages 390-391). Thus, transformation with genes encoding cold-tolerant enzymes would not affect the accumulation of sucrose in cold storage. Therefore, claim 19 should be limited to the exemplified phosphofructokinase gene, given the lack of guidance in the specification regarding the identification or isolation of other genes, the unpredictability inherent in the survival and health of plants transformed with a variety of glycolytic genes as discussed supra. and the limited cold sensitivity of the enzymes as discussed supra."

It is submitted however, that Ap Rees et al wished to test the hypothesis that the cold-lability of PFK is a major cause of sweetening. They used a potato (13737) in respect of which their data indicated that the four forms of PFK were less cold-labile than the PFK of record. They then found that after cold storage 13737 had sweetened less than Record. Thus, to a skilled-in-the-art addressee who possessed an interest in reducing sweetening during

cold storage, the message is to identify varieties of the PFKs which exhibit comparatively low levels of cold-lability.

In the Ap Rees et al paper there is no pointer whatsoever to the introduction of additional PFK. As Professor Ap Rees himself said in his declaration, (copy attached), the paper is devoid of a teaching, even an implicit one, that a low level of cold storage sweetening can be obtained if potatoes are transformed with a sequence coding for PFK. Professor Ap Rees makes the point that this is the case even if the paper is read in the light of common general knowledge as of December 1989.

In any event, it is well settled that patent applications need not provide detailed examples of every species encompassed by a generic claim, even in an unpredictable art; see In re Angstadt, 190 USPQ 214. In the present claims, the success with 4 enzymes of 8 supports a conclusion that the 8 are enabled. The enzymes are all recognized as affecting glycolysis in plant cells. Reconsideration of the rejection is requested.

Clearly, Applicants have met the mandate of 35 USC 112, first paragraph, which is to teach how to "make and use" the invention. The enablement requirement need not be met with specific working examples (In re Borkowski, 164 USPQ 642) but by consideration of the specification as a whole. The question of predictability has been answered

in applicants' favor by the appearance of the von Schaewen publication. the claims are process claims and the products of those processes. Practice of these processes do not require undue experimentation. There is "insufficient unpredictability" within the meaning of the Board of Patent Appeals and Interferences' decision in the case of Ex Parte King, 17 USPQ 2nd 1545. Those skilled in the art with the evidence shown by the actual use of the genes for the 4 expressed enzymes and knowledge of the metabolic pathways shown in the drawing of Applicant's Fig. 1, would expect the operative effect of transgenesis with the remaining 4 enzymes.

Claim 7 stands rejected under 35 U.S.C. 102(b) as being anticipated by Grill et al. (WO 89/08145). Reconsideration of the rejection is requested for the following reasons.

Claim 7 is narrower in scope than Claim 2 being dependent thereon and directed to the embodiment wherein the host plants are specifically named plants. The Grill et al. reference does not show or suggest the process of Claim 2 and Claim 7 is patentably distinguished for the same reasons given above.

Claim 8 is rejected under 35 U.S.C. 103 as being unpatentable over Grill et al. (WO 89/08145). Reconsideration of the rejection is requested for the following reasons.

Claim 8 is narrower in scope than Claim 7, from which it depends being directed to the embodiment wherein the host plant is a potato of specified variety. It is submitted that Claim 8 is patentably distinguished from the cited reference for the same reason given above to distinguish Claim 7. the cited prior art reference does not show or suggest the process of Claim 2 from which both claims depend.

Claims 2-4, 7, 13-16 and 21-24 stand rejected under 35 U.S.C. 103 as being unpatentable over Twell et al. taken with de Graaff et al., Ap-Rees et al. and Yang et al. Reconsideration of the rejection is requested for the following reasons.

Claim 2, the method claim of broadest scope, is directed to a method for the preparation of a transgenic plant. In accordance with the method, a plant cell is transformed with a chimaeric gene comprising a promoter and a gene encoding for a polypeptide having the activity of an enzyme which regulates the amount of a metabolic intermediate in glycolysis or in a pathway for the synthesis or degradation of starch, sucrose or reducing sugar from a glycoyltic intermediate.

For example, in a transgenic potato plant embodiment of the invention, an increased level of pyruvate kinase results in reduced accumulations of sugars in the tubers of the plant. Until the present invention, it was thought

that PFK alone controlled the total glycolytic flux. However, applicants found that this is not the case. Applicants introduce pyruvate kinase activity into potato plants by genetic manipulation. The results indicate that a substantial increase in pyruvate activity does not substantially alter flux through glycolysis but changes the pool sizes of intermediates; see the experimental data set forth in Applicants' Example. This indicates that regulation of glycolytic flux may be achieved not only at the entry of carbon into the pathway but also by exit from it.

It is submitted that none of the cited prior art references, alone or in any combination, show or suggest the invention of Claim 2 and the unexpected findings described above. More particularly, Twell et al. teaches that the potato may be transformed with the tuber specific patatin promoter and a chimaeric gene for patatin. As recognized by the Examiner, Twell et al. is not concerned with and does not suggest any other gene or its expression in a transgenic plant.

deGraaf et al. is cited only for the disclosure of the cloning of a fungal pyruvate kinase gene and its expression in a host.

Ap-Rees et al. teaches the relationship between sucrose accumulation in potato tuber and loss of PFK activity and the undesirability of sucrose accumulation in

cold-stored potato. The reference has been discussed above and distinguished from the present invention.

Yang et al. is cited for their teaching of "transformation to alter potato tuber quality" and their suggestion of the patatin promoter (the patatin promoter was suggested, but not used, as possibly improving the yields of protein which might be expressed by the DNA sequence coding for a protein high in essential amino acids). Nothing was suggested concerning carbohydrate metabolism.

Based on the four cited references, it was concluded in the Office Action that:

It would have been obvious to one of ordinary skill in the art to utilize the method of potato transformation taught by Twell et al., and to modify that method by incorporating the pyruvate kinase structural gene taught by deGraaff et al.; given the teaching by Ap-Rees et al. of the relationship between tuber sweetening and pyruvate kinase activity, the suggestion by Yang et al. to utilize plant transformation for potato tuber quality improvement, and the recognition by those of ordinary skill in the art that each would have continued to function in its known and expected manner. Thus, the claimed invention was clearly prima facie obvious as a whole to one of ordinary skill in the art at the time it was made, especially in the absence of evidence to the contrary."

In other words, Twell et al. and deGraaff et al. are representative of the technique for preparing transgenic plants. Applicants agree that the techniques of preparation are indeed "state of the art". However, obviousness under 35 USC 103 can only be determined when

the prior art is considered as a whole, including the differences between the prior art and the claimed invention. In the decision of the Federal Circuit Court of Appeals rendered in Northern Telecom, Inc. V. Datapoint Corp., 15 USPQ 2d 1321 the court found that the nature of the problem which persisted in the prior art and the inventor's solution to the problem must be considered in determining prima facie obviousness.

Thus, although the techniques of preparing a transgenic plant are known, where does the cited prior art suggest that the process of the invention will solve, for example, the problem of sugar accumulation in the tuber of a potato plant?

As mentioned above, Ap-Rees does not concern transgenic plants and does not equate PFK activity to sugar accumulation in plants at harvest. Ap-Rees concerns cold-lability of the PFK forms. Ap-Rees offers no motivation to reduce sugar accumulations by enhancement of PFK activity by any means whatsoever, especially by genetic manipulation.

Yang et al. does not fill the void of Ap-Rees. The Yang et al paper is merely representative of the common general knowledge. All it provides is an exemplification of the fact that techniques are available whereby genes can be transferred into plants. The commonality of plant type (potato) between Yang et al and Ap Rees et al is, in

the present context, of no import. The examiner mentions the fact that Yang et al were seeking to modify plant quality. This is a feature of all commercially orientated work involving the genetic modification of plants. In other words, the citation of Yang et al merely exemplifies plant improvement by genetic manipulation.

Furthermore, as stated by Judge Rich in the decision of *In re Vaeck*, 20 USPQ 2d 1438

"Where claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under Section 103 requires, inter alia, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should *** carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. [citations] Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicants' disclosure."

(underlining added)

Both of the required elements are lacking in the prior art cited in the application at bar, particularly in respect to an expectation of a solution of the prior art problem.


The invention is particularly applicable to potatoes. It had been expected that the introduction and expression of additional pyruvate kinase into potato tuber cells would cause a high flux in the glycolytic pathway. Flux

is defined as is defined as the rate at which chemical compounds are converted from one compound to another in a pathway. A metabolic intermediate is a chemical compound in the pathway which is converted into something else (metabolism being the process of converting one chemical into another).

For all of the above reasons, it is submitted that claims 2-4, 7, 13-16 and 21-24 are all patentably distinguished from the cited prior art and allowable.

A speedy and favorable reconsideration of the rejection is requested, together with new claims 27-30.

Respectfully submitted,



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